Received: 21 March 2023 | Accepted: 8 June 2023

DOI: 10.1111/jfb.15479

REGULAR PAPER

JOURNAL OF FISH BIOLOGY fsbi

Andrology of shortnose guitarfish *Zapteryx brevirostris* (Müller & Henle, 1841) (Chondrichthyes, Trygonorrhinidae)

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Abstract

The andrological study of a species involves the macro- and microscopic analyses of the internal reproductive organs and the evaluation of seminal parameters and ultrastructural characteristics of the spermatozoa. As in other vertebrates, the male reproductive tract in chondrichthyans consists of testes and reproductive ducts (efferent duct, epididymis, Leydig's gland, ductus deferens and seminal vesicle). In this study the authors used three adult specimens of *Zapteryx brevirostris* from wild capture kept at the Ubatuba Aquarium, Brazil. Semen was collected by abdominal massage over the location of the seminal vesicle, preceded by ultrasonographic evaluation. The semen collected was diluted 1:200 and subject to quantitative and morphological analyses. Ultrastructural analysis was performed using transmission and scanning electron microscopy. Correlation was observed between successful collection and ultrasonographic image of an engorged seminal vesicle, as well as testicles with easily delimitable margins and higher echogenicity. It was possible to identify free spermatozoa with helical filiform appearance, as well as spermatozeugmata. The average sperm concentration resulted in 5 million packets per millilitre and 140 million spermatozoa per millilitre. The sperm nucleus is described as follows: cone shaped, parachromatin sheath less dense than the chromatin of the nucleus, smooth depression of the nuclear fossa, abaxial axoneme 9 + 2 and accessory axonemal columns in positions 3 and 8 and oval shaped, with flattened inner surface in cross-section. These results broaden the knowledge of the andrology of this species, contributing to *ex situ* breeding programmes.

KEYWORDS
conservation, elasmobranchs, reproduction, semen, spermatozeugmata, spermatozoa

1 | INTRODUCTION

Shortnose guitarfish, *Zapteryx brevirostris* (Müller & Henle, 1841), is the only species of the genus *Zapteryx* found in the western Atlantic Ocean. Its geographic distribution ranges from south-eastern Brazil to Mar Del Plata, Argentina (Gomes *et al*., 2019). The species is described as "threatened" in the IUCN Red List and in the Brazilian list of threatened species (MMA Federal Department of the Environment, Ordinance No. 148, of 7 June 2022) (MMA, 2022). This is due to the fact that shortnose guitarfish is categorized as "k strategist," and it is captured as by-catch in artisanal fisheries (Bornatowski *et al*., 2009; Costa & Chaves, 2006; Maganhe *et al*., 2022; Robert, 2012).

The maximum total length (TL) reported for females of *Z. brevirostris* is 661 mm compared to 635 mm for males

(Castello, 1971). This species attains its first sexual maturity stage when 420 mm TL is reached for females and 437 mm TL for males. At 470 and 450 mm TL, all females and males, respectively, are 100% mature (Batista, 1987a). The sex ratio can vary from 1:1 (male: female) (Batista, 1987b; Colonello *et al*., 2011) to 1:17 (Santos *et al*., 2006).

The internal organs of the reproductive system of male elasmobranchs include the testes, genital ducts, Leydig's gland, alkaline gland and clasper sac (Hamlett, 1999). The path of spermatozoa in rays and sharks can be simplified as follows: from the efferent ductules, they swim through the highly convoluted epididymis that are closely associated with the ventral surface of an accessory organ called the Leydig's gland. The sperm undergo a period of maturation in the epididymis before reaching the ductus deferens, which gradually enlarges to form the seminal vesicles. The seminal vesicles then join, resulting in the urogenital papilla, which, in turn, empties into the cloaca. At copulation and ejaculation, the spermatozoa are transferred from the seminal vesicle, through the urogenital papilla, to the dorsal groove of each clasper.

Semen analysis, as a standard laboratory test, provides basic information on spermatogenesis, secretory activity of the gonads and reproductive health of the male. The results obtained during such analysis can indicate either the absence of spermatozoa or severe or mild deviations in sperm parameters, as well as normal values of semen volume, sperm count and concentration, motility and morphology. That is, semen parameters are relevant both in natural conception and in the results of assisted reproduction technologies (Sharma *et al*., 2020).

According to Mattei (1991), it is not possible to construct a spermatic model for the fishes, as is the case, *e.g*., for mammals and snakes. The ultrastructure of the gamete generally depends on the method of fertilization and on the evolutionary lineage to which the fish belongs (Mattei, 1991). In some cases an evolved spermatic form is found only in a certain group: a family, a suborder, an order, a superorder or a class (Mattei, 1991). Therefore, the results of sperm ultrastructure can provide valuable information on cellular modifications associated with reproductive habits and reveal morphological characters useful for hypothesizing phylogenetic relationships (Baicere-Silva *et al*., 2011; Burns *et al*., 2002).

Sustaining viable populations of all wildlife species requires the maintenance of habitat, as well as an understanding of the anatomy, physiology and behaviour of individual species. *Ex situ* recovery programmes that include reproductive studies are the usual first line of defence when species become critically endangered (Pukazhenthi *et al*., 2005). The management of animal reproduction in captive populations and their reintroduction/release into the wild and other approaches such as assisted reproductive technologies and biobanking greatly contribute to the success of conservation breeding.

Being aware that in public aquaria there is still little engagement regarding the sustainability of the animal stock (Buckley *et al*., 2018; Daly & Jones, 2017; García-Salinas *et al*., 2021a; Henningsen *et al*., 2017) and given the threatened status of *Z. brevirostris*, it is clear that there is a need to develop strategies for *ex situ* conservation programmes of this species, through the conservation of its spermatozoa and the application of assisted reproduction techniques. The first steps for the development of the techniques include knowledge of the macro- and microscopic anatomy of the reproductive system, as well as semen collection and characterization (Wyffels *et al*., 2021). The aim of this study was to identify the andrological parameters of the species, through macro- and microscopic observation of the reproductive organs, and to perform sperm evaluation, including ultrastructural examinations of the spermatozoa of *Z. brevirostris*.

2 | MATERIALS AND METHODS

2.1 | Animals

Three males of shortnose skate (*Z. brevirostris*) kept at the Ubatuba Aquarium (Ubatuba, São Paulo, Brazil; latitude, 23° 26′ 47.71″ S; longitude, 45° 4′ 5.21″ W) from incidental capture in the shrimp *Xiphopenaeus kroyeri* fishery (Heller, 1862) were used in this study. These three individuals were kept with three females of the same species, in a 5.2 m^2 tank, without substrate, under recirculation, coupled to an 80,000 l system, equipped with two sand filters, ozone injection, biological filter, ultraviolet lamps, nitrate filter, skimmer and chiller. The physical and chemical parameters of the water were monitored twice a week using the commercial Hanna probe model HI 98194 and, whenever necessary, corrected to maintain the salinity at 31, temperature at 22°C and pH at 8.2. Nitrogen levels were measured using commercial tests (Alcon), respecting the limits recommended by Mohan and Aiken (2004). The animals were also subjected to a 12–12 h photoperiod regime and a diet based on frozen seven barb shrimp (*ad libitum* consumption).

In the previous biometry, the TL of males was 500, 470 and 459 mm; disc width was 218, 212 and 215 mm; and weight was 858, 770 and 65 g, respectively. All specimens were mature according to Batista (1987a). On palpation, they presented rigid claspers.

2.2 | Organ anatomy and histology

After the death of one of the males, a necropsy was performed at the Ubatuba Aquarium. With the carcass positioned in dorsal decubitus, a U-shaped incision for access to coelomic cavity was made ventrally.

For histological analysis, the complete reproductive tract of the male *Z. brevirostris* was removed, sectioned into 0.5 cm slices, fixed in 10% formalin for 24 h and later preserved in 70% ethanol.

The sample was dehydrated in ethanol series at increasing concentrations (from 70% to 100%) and diaphanized in xylol, with subsequent inclusion in paraplast. The material was then cut, using a Leica RM 2065 microtome, into 3 μm thick fragments, which were then adhered to glass slides. These slides were kept in an oven at 60°C for 24 h and then passed through batteries of xylene and alcohol for haematoxylin and eosin (HE) staining. A replica was subjected to Masson's trichrome staining. The slides were read under a trinocular microscope

(ZEISS – Axiolmager.A2.m) at the Centro Avançado em Diagnóstico por Imagem (São Paulo, São Paulo) – CADI – FMVZ-USP.

2.3 | Ultrasound

The other two males were removed from the tank and kept – one at a time – in a plastic box (540 mm length × 330 mm width × 120 mm height) containing 10 l of water from the tank of origin and provided with aeration. Before sperm collection, with the specimens in dorsoventral position, an ultrasound examination was performed using a Mindray M6 equipment, coupled to a 3.5 MHz convex/linear transducer, aiming to confirm the presence or absence of semen in the seminal vesicle and to evaluate the testes.

2.4 | Semen collection

After the imaging exam, tonic immobility was induced by keeping the two specimens in ventrodorsal position and applying light pressure to the rostrum. The cloaca emerged and was dried with a paper towel. A gentle bilateral pressure in the abdominal region, over the location of the seminal vesicle, was enough to make the sperm flow through the urogenital papillae. Semen was collected directly into 15 ml polypropylene falcon centrifuge tubes. The sample was then subjected to analysis using optical microscopy, scanning electron microscopy (SEM) and transmission electron microscopy (TEM).

2.5 | Semen evaluation

Immediately after collection, semen was evaluated for macroscopic (colouration, appearance and volume) and microscopic (motility, vigour and sperm morphology) parameters; 10 µl of semen was allocated between slide and coverslip, preheated at 37°C and observed under a light microscope (Binocular Microscope – N 107 Coleman) to validate the presence of spermatozoa, as well as the measurement of sperm motility and vigour. Magnifications of 40×, 100× and 400× were used. The semen was diluted 1:200, and the sperm concentration was calculated in a Neubauer chamber. Sperm morphology was analysed using two different methodologies: (a) smear of semen stained with Panotic stain and (b) preparation in a humid chamber, with 1:20 dilution in formalin saline.

A sample was also dehydrated in ethanol series, in increasing concentrations (from 70% to 100%), and diaphanized in xylol, with subsequent inclusion in paraplast. Subsequently, the material was cut, using a Leica RM 2065 microtome, into 3 µm thick fragments, which were then adhered to glass slides. These slides were kept in an oven at 60°C for 24 h and then passed through batteries of xylene and alcohol for HE staining. The slides were read under a trinocular microscope (ZEISS – Axiolmager.A2.m) at the Centro Avançado em Diagnóstico por Imagem (São Paulo, São Paulo) – CADI-FMVZ-USP.

2.6 | Scanning electron microscopy

For this analysis, the samples were (a) fixed in 4% paraformol; (b) dehydrated in increasing series of alcohols in concentrations of 70%, 80%, 90% and 100%; (c) dried in a LEICA EM CPD 300 critical point apparatus; (d) glued with carbon glue on aluminium metallic bases (stub); (e) sputtered with gold in an EMITECH K550 metallizer and, finally, (f) analysed and photo-documented under a LEO 435 VP scanning electron microscope at the Centro Avançado em Diagnóstico por Imagem (CADI-FMVZ-USP).

2.7 | Transmission electron microscopy

Samples were prepared for TEM as follows: (a) spermatozoa pellets were fixed in a solution of 2.5% glutaraldehyde in 0.15 M phosphate buffer, pH 7.2, for 2 h; (b) post-fixed in 1% osmium tetroxide solution in saline, contrasted in 0.5% uranyl acetate solution in saline, for 12 h; and (c) embedded in Araldite resin. (d) The ultra-thin sections (70 nm) were subjected to double contrasting using 2% uranyl acetate and 0.5% lead citrate and finally (e) analysed using a Morgagni 268D transmission electron microscope at the Centro Avançado em Diagnóstico por Imagem – CADI-FMVZ-USP.

2.8 | Code of ethics

This study was approved by SMA/SP AM2856440 and Ethics Committee in the Use of Animals of the Ubatuba Aquarium (no.04/2021). This study is justified by the need to expand knowledge about the reproduction of elasmobranchs in captivity. The minimum possible number of individuals needed to obtain the information was used.

3 | RESULTS

Necropsy allowed the authors to evaluate the location and macroscopic aspect of the organs of the reproductive system (Figure 1). The testis had lobulations on its dorsal surface, visible to the naked eye, and limitation of the epigonal to its extremity. Epididymis and ductus deferens were highly convoluted. The seminal vesicle was already observed even before the liver was removed from the coelomic cavity, with a size and conformation that easily allowed its differentiation from the ductus deferens. The glands of the clasper were easily differentiated from the surrounding tissue and were pale yellow. The length of the claspers exceeded that of the pelvic fins.

The organs of the male reproductive system were evaluated for histological architecture (Figure 2). The ducts of the testis, epididymis, seminal vesicle and Leydig's gland had a simple ciliated columnar epithelium. The epithelium of the alkaline gland consisted of tall columnar cells without cilia. The clasper gland comprised multiple simple tubular secretory units arranged radially relative to the ventral groove, where the papilla was located; each glandular unit had a tall simple

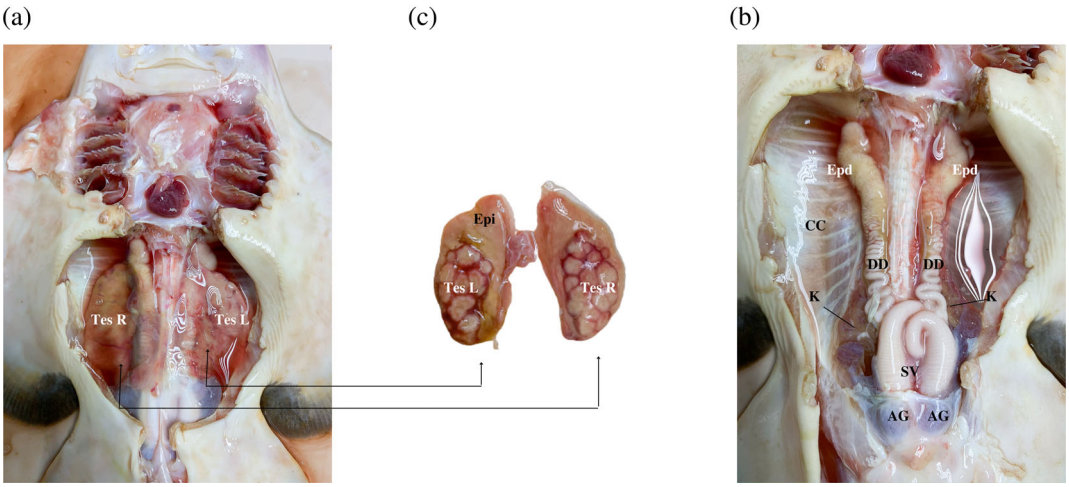

FIGURE 1 Necropsy of male shortnose guitarfish (*Zapteryx brevirostris*). (a) Ventral view of the coelomic cavity after removal of the skin, musculature, liver and gastrointestinal tract. (b) Ventral view of the sexual organs in the coelomic cavity after removal of the testicles. (c) Dorsal view of the testicles. AG, alkaline gland, CC, coelomic cavity; DD, ductus deferens; Epd, epididymis; Epi, epigonal; k, kidney; SV, seminal vesicle; Tes L, left testis; Tes R, right testis.

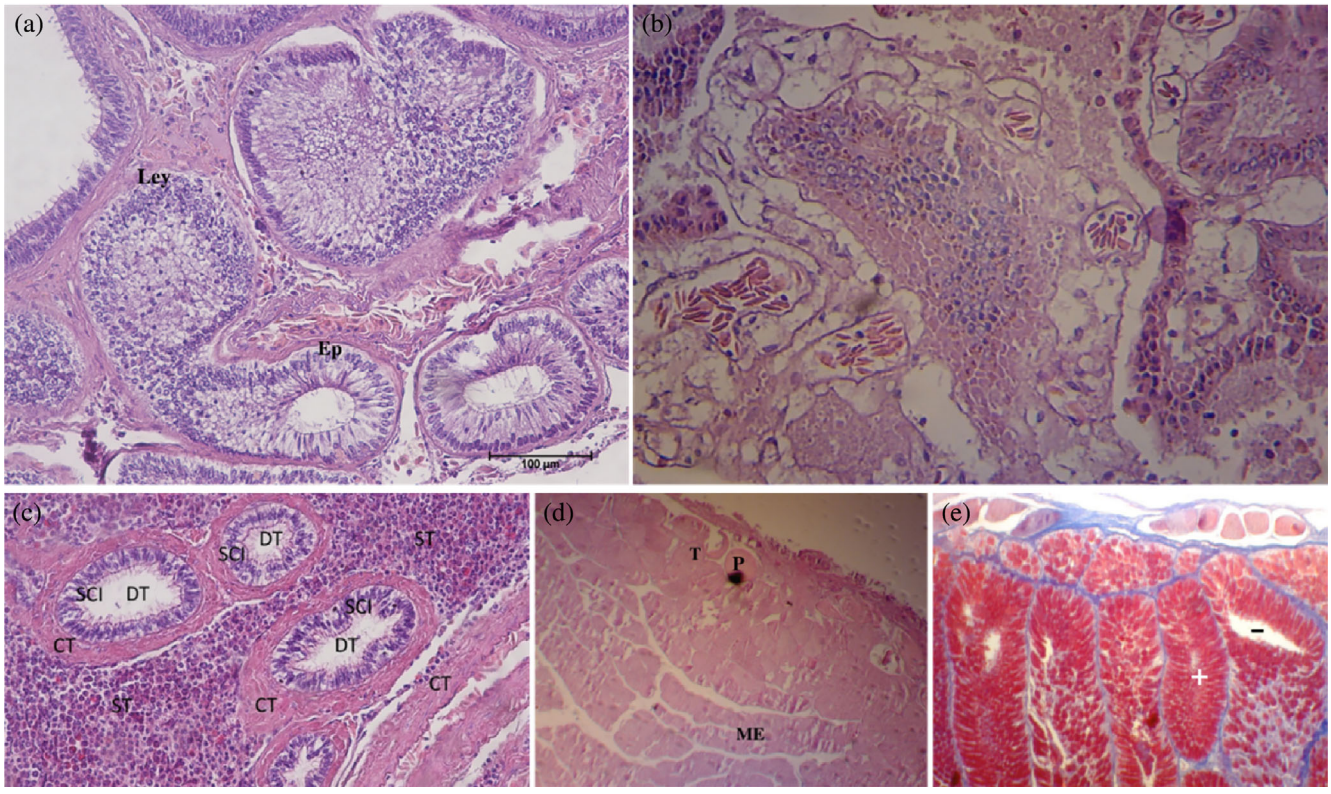

FIGURE 2 Reproductive system of the male shortnose guitarfish (*Zapteryx brevirostris*). (a) Histological architecture of the Leydig's gland and epididymis of mature individual [HE (haematoxylin and eosin) stain, 100×]. (b) Histological architecture of the alkaline gland of mature individual (HE stain, 100×). (c) Histological architecture of the testis of mature individual (HE stain, 100×). (d) Histological architecture of the clasper gland of mature individual (HE staining, 40×). (e) Histological architecture of the clasper gland of a mature individual, showing secretory tubules with abundant luminal secretion (+) and others without accumulated secretion (−) (Masson's trichrome stain, 100×). CT, connective tissue; DT, secretory duct; Ep, epididymis; Ley, Leydig's gland; ME, striated muscle; P, papilla; SCI, primary spermatocytes; T, tubules or glandular units

columnar epithelium composed of secretory cells, with basal nuclei and cytoplasm filled with eosinophilic granules. The epididymis had a large and vascularized lumen, with abundant amounts of matrix forming a dense fluid, with clusters of spermatozoa in the form of spermatozeugmata. Spermatozoa were also visualized in the lumen of the ducts of the testis and seminal vesicle (Figure 3).

Ultrasound was performed with the transducer in transverse orientation, cranial to the pelvic girdle, revealing oval-shaped sections of the seminal vesicle. The sonographic image of the heterogeneous hypoechoic engorged centres may indicate the presence of seminal content (Figure 4). Imaging examination also indicated seasonal variation in testis size: the right and left testes in both animals presented higher values of length and width, as well as higher echogenicity – and pixel intensity of the image – at the end of April when compared to the images taken at the end of June (Figure 5).

Ejaculates were collected from two males on 28 April 2022. The samples had the following seminal parameters: ivory colouration, creamy appearance and a total volume of 0.9 ml. Observation of the semen under a light microscope revealed that many of the spermatozoa were tightly packed in the region of the cephalic portion (head) (Figure 6). It was not possible to accurately determine progressive sperm motility due to the concomitant presence of sperm packets and free sperm. It was possible to observe that spermatozoa were in a motile state in the fresh (or pure) semen. The spermatozoa that were free had a helical filiform appearance. The average sperm concentration resulted in 5 million packets per millilitre and 140 million sperm per millilitre. The cephalic portion (or head), the midpiece and the tail (or flagellum) (Figure 7) were visualized using the Rapid Panoptic stain and wet slides.

Circular masses of spermatozoa were observed using SEM, in which the sperm heads were aligned side by side and embedded in a seminal matrix, whereas their tails extended outward (Figure 8). In *Z. brevirostris*, the midpiece is shorter but has greater width, when compared to the head. Moreover, it was observed that the midpiece in this species is not helical.

Analysis of the TEM results revealed that the nucleus of the *Z. brevirostris* spermatozoon is cone shaped, pointed at the anterior end. Its dense chromatin is surrounded by a parachromatin sheath of lower density (grey colour). The depression of the nuclear fossa is smooth.

The midpiece appears to consist of a cluster of approximately isodiametric mitochondria, having as components a central axial rod, which fits into a recess at the posterior end of the nucleus, whereas its posterior end inserts into the basal body (distal centriole) of the flagellum. The midpiece is surrounded by a fibrous sheath that overlaps the posterior end of the nucleus. A cytoplasmic sleeve is present, functioning as a sleeve that covers the midpiece and the flagellum.

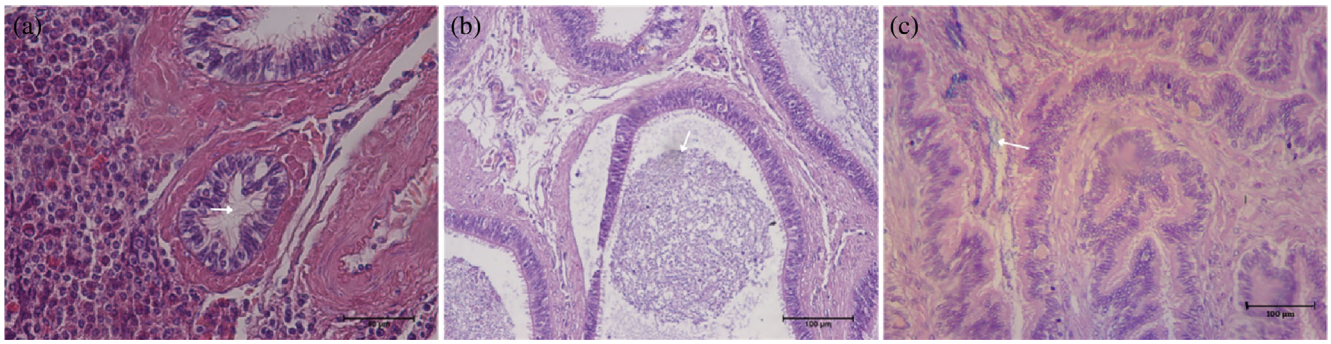

FIGURE 3 Histological architecture of the testis (a), epididymis (b) and seminal vesicle (c) of a mature individual shortnose guitarfish (*Zapteryx brevirostris*). White arrows indicate the presence of free or packaged spermatozoa [HE (haematoxylin and eosin) stain, 100×]. Scale: 100 μm

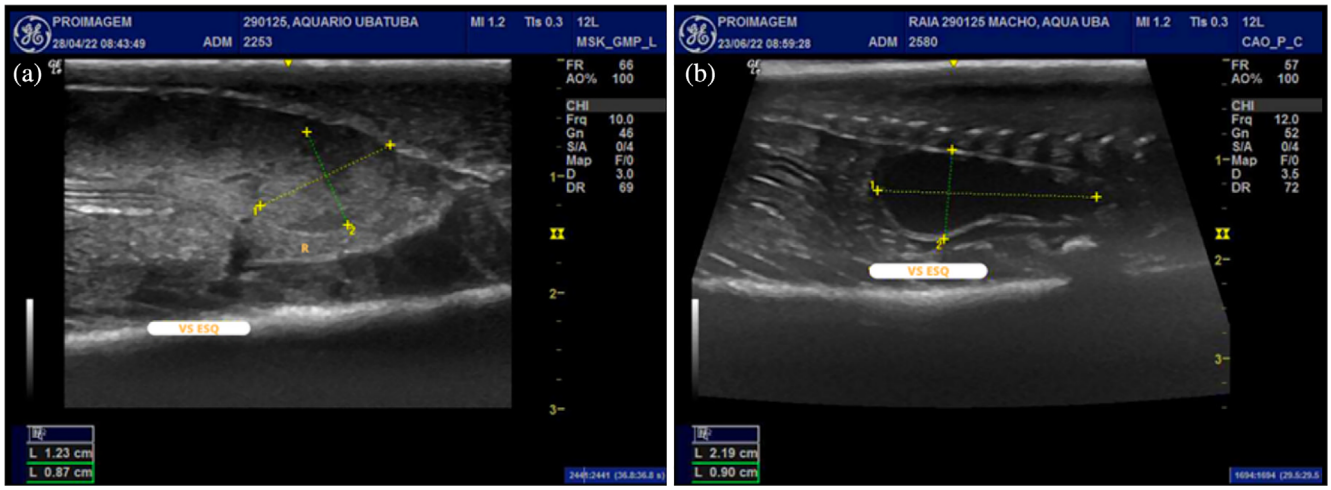

FIGURE 4 Ultrasonographic evaluation of the seminal vesicle of shortnose guitarfish (*Zapteryx brevirostris*) on (a) 28 April 2022 and (b) 23 June 2022. The seminal vesicle content is hypoechoic when compared to the kidney (R)

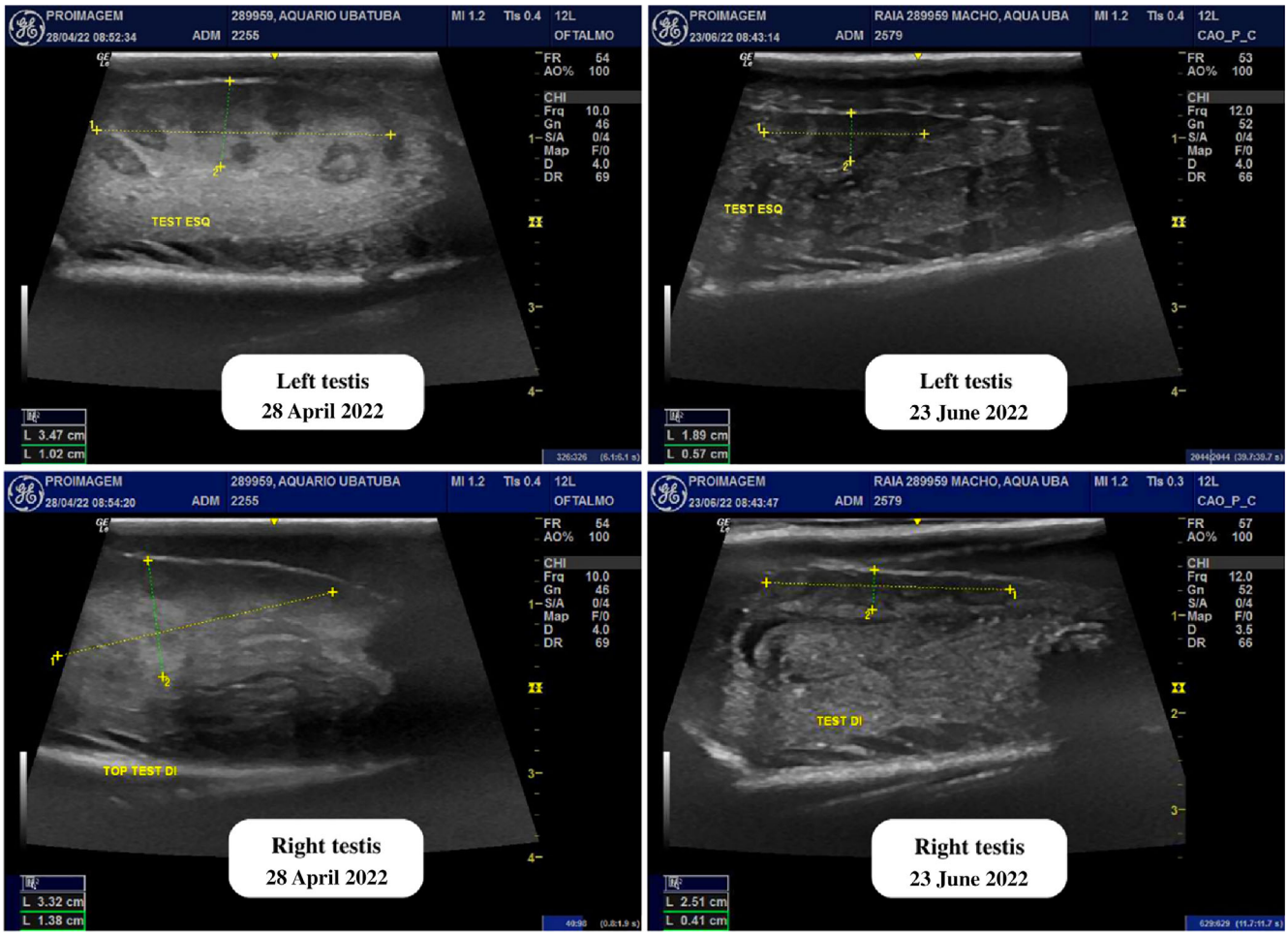

FIGURE 5 Ultrasonographic evaluation of right and left testicles of shortnose guitarfish (*Zapteryx brevirostris*) comparing two dates. Both testes were larger in terms of length and width, as well as greater echogenicity, in late April

The tail, originating from the posterior end of the midpiece, contains an axoneme 9 + 2, abaxial, accompanied by two longitudinal columns (accessory axonemal columns). These longitudinal columns, in turn, are in positions 3 and 8 and present an oval shape in cross-section, with a flattened inner surface (Figure 9).

4 | DISCUSSION

Due to the rigidity of the clasper, confirmed by simple palpation of the organ, added to the visualization of spermatozoa on histological slides made from sections of the seminal vesicle, it can be concluded that the male that died had already reached sexual maturity. Therefore, the morphological description of the organs – found in this study – is efficient to classify if a certain *Z. brevistris* individual is an adult.

The compressed morphology of the testis in *Z. brevirostris* is typical of Batoidea. The epigonal organ is mainly limited to the tip of the testis, because the epigonal to germinal testicular tissue ratio decreases throughout the stages of sexual maturity, due to the development of this germinal testicular tissue. Such development also enables the lobular nature of the outer surface of the organ to be visible to the naked eye (Basallo *et al*., 2018).

Reproductive ducts are highly convoluted in adult *Z. brevirostris* and in most elasmobranch species. An exception is the species *Gymnura poecilura* (Shaw, 1804), in which the absence of coiling results in considerably shorter organs. Such a finding may be related to the fact that sperm remain in free, non-aggregated form throughout the reproductive system of the male of *G. poecilura* (Henderson *et al*., 2014).

In immature males, the epididymis appears as a virtually closed duct with a narrow lumen and no spermatozoa, whereas in mature males, the organ is larger and vascularized, with abundant amounts of seminal matrix forming a dense fluid with clusters of spermatozoa (Basallo *et al*., 2018). The latter pattern was the one observed in the necropsied male of *Z. brevirostris*. Also in an immature elasmobranch, the epithelium of the Leydig's gland tubules tends to show little or no secretory activity, as reported by Basallo *et al*. (2018) in *Sympterygia acuta* (Garman, 1877) and *Sympterygia bonapartii* (Müller & Henle, 1841), and different from that found for *Z. brevirostris* in this study.

The epithelium in the alkaline gland in *Z. brevirostris* observed under a light microscope showed the same characteristics reported

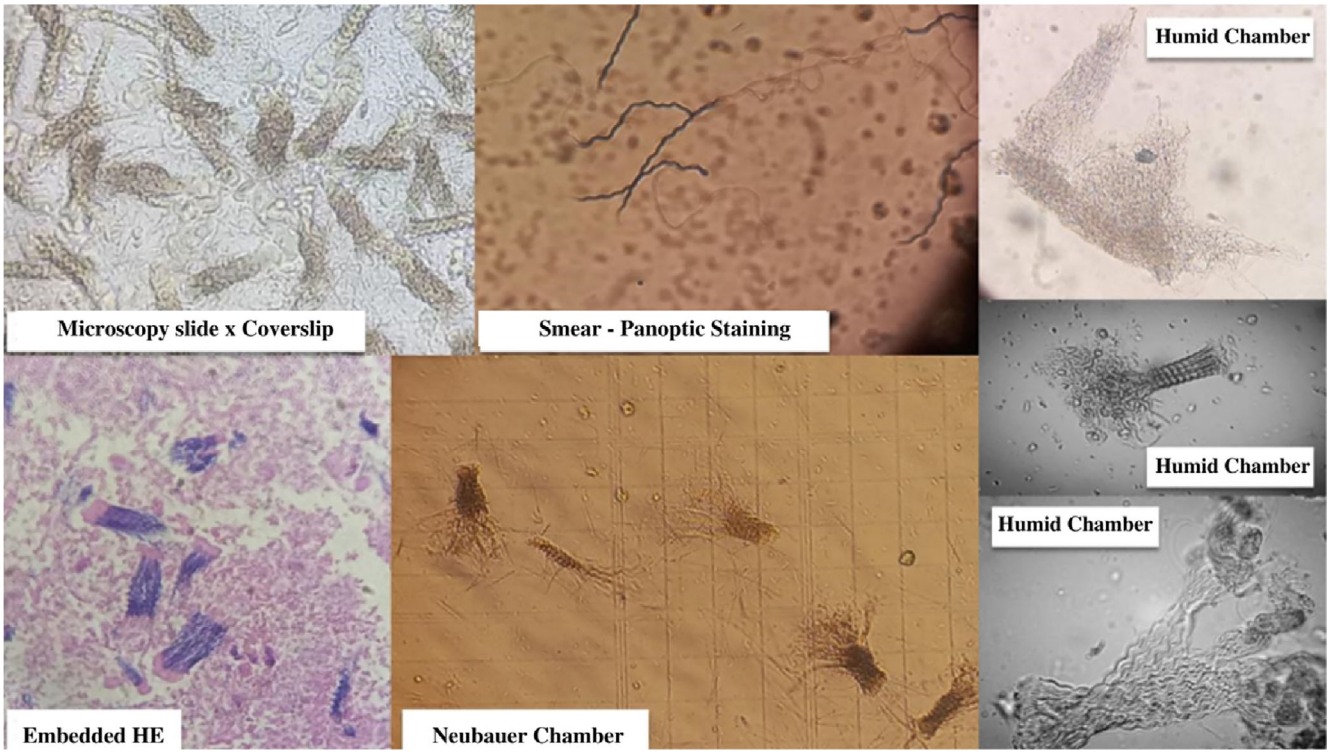

FIGURE 6 Analysis of sperm morphology in shortnose guitarfish (*Zapteryx brevirostris*) using different techniques. Free and packaged spermatozoa are observed

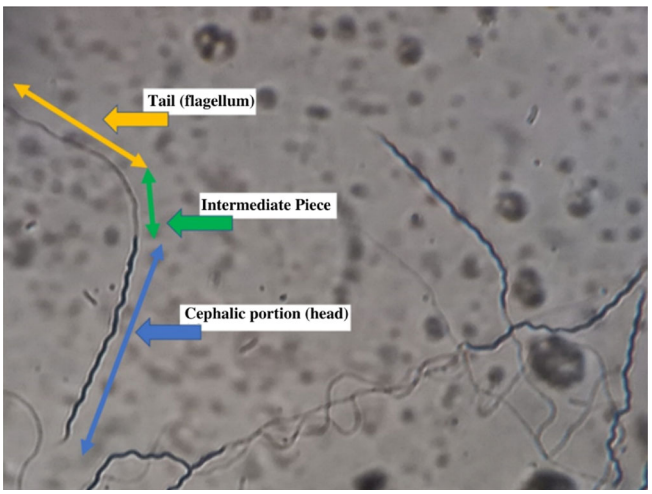

FIGURE 7 Shortnose guitarfish (*Zapteryx brevirostris*) spermatozoa evaluated from semen smear stained with Panotic stain. The three main components were highlighted: cephalic portion (or head), midpiece and tail (or flagellum)

for *Raja erinacea* (Mitchill, 1825) (Hamlett, 1999) and *G. poecilura* (Henderson *et al*., 2013). Although the main function of the gland is still being discussed, it is known that its secretions have a positive effect on the activation of the sperm, thus increasing spermatozoa motility (García-Salinas *et al*., 2021b; Hug *et al*., 2000).

In the necropsied individual, the clasper gland was observed to be developed. According to Anaya-López and Ramírez-Pinilla (2017), the size of the clasper gland varies significantly between mature juvenile and immature males. In histology, the fact that the gland presents secretory tubules with abundant luminal secretion and others without accumulated secretion is suggestive of an asynchrony in the secretory process of the organ.

In this study, sperm collection was preceded by imaging. A positive correlation was established between semen collection and sonographic imaging of the engorged seminal vesicle. Such methodology was also successfully implemented for sharks of the species *Carcharias taurus* (Rafinesque, 1810) (Wyffels *et al*., 2020). Additionally, an evaluation of the testes showed greater size and echogenicity when the seminal vesicle was engorged and, concomitantly, when semen collection was possible. That is, ultrasonographic evaluation is useful for the analysis of testicular activity (spermatogenesis) in *Z. brevirostris*.

Semen collection in elasmobranchs can occur *via* abdominal massage or by using a cannula or catheter, inserted through the urogenital papilla (García-Salinas *et al*., 2021b; Wyfels *et al*., 2021). Both methods require knowledge of the anatomy of the species being managed. Collection *via* massage was the option chosen given its applicability and lower risk of injury to internal structures, because the shortnose guitarfish, being a small elasmobranch, has a considerably reduced papilla diameter.

Although it was not possible to accurately determine progressive sperm motility due to the concomitant presence of free and packaged sperm, it was visualized that fresh (or pure) semen from *Z. brevirostris* already had sperm in a motile state. This observation corroborates

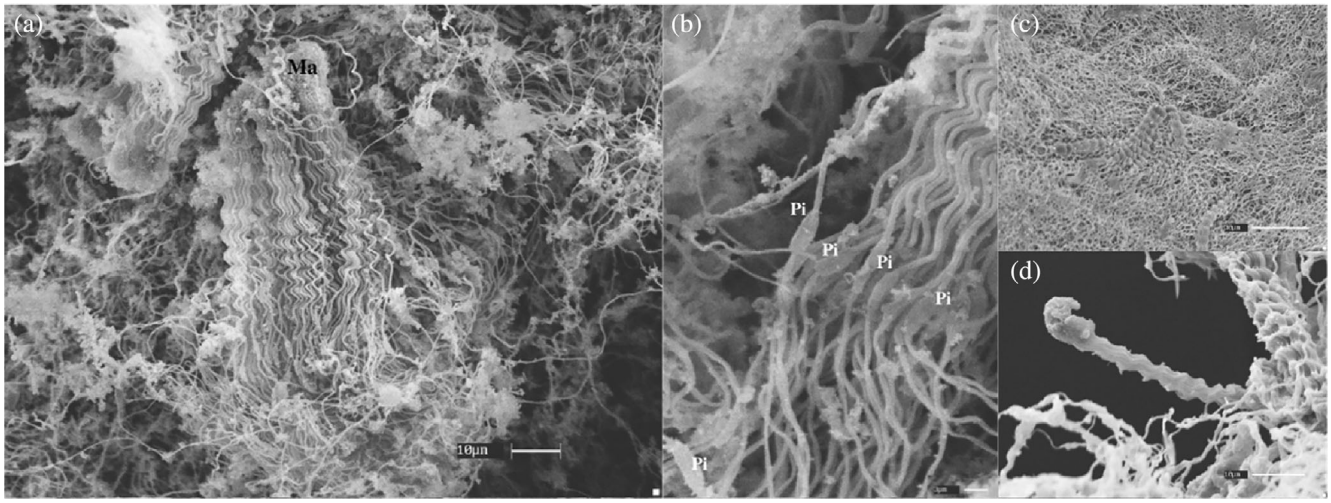

FIGURE 8 Scanning electron microscopy images taken from the shortnose guitarfish (*Zapteryx brevirostris*) semen sample, confirming the presence of spermatozeugmata. Ma, matrix; Pi, intermediate piece. Scale in (a) and (d): 10 µm; scale in (b): 3 µm; scale in (c): 30 µm

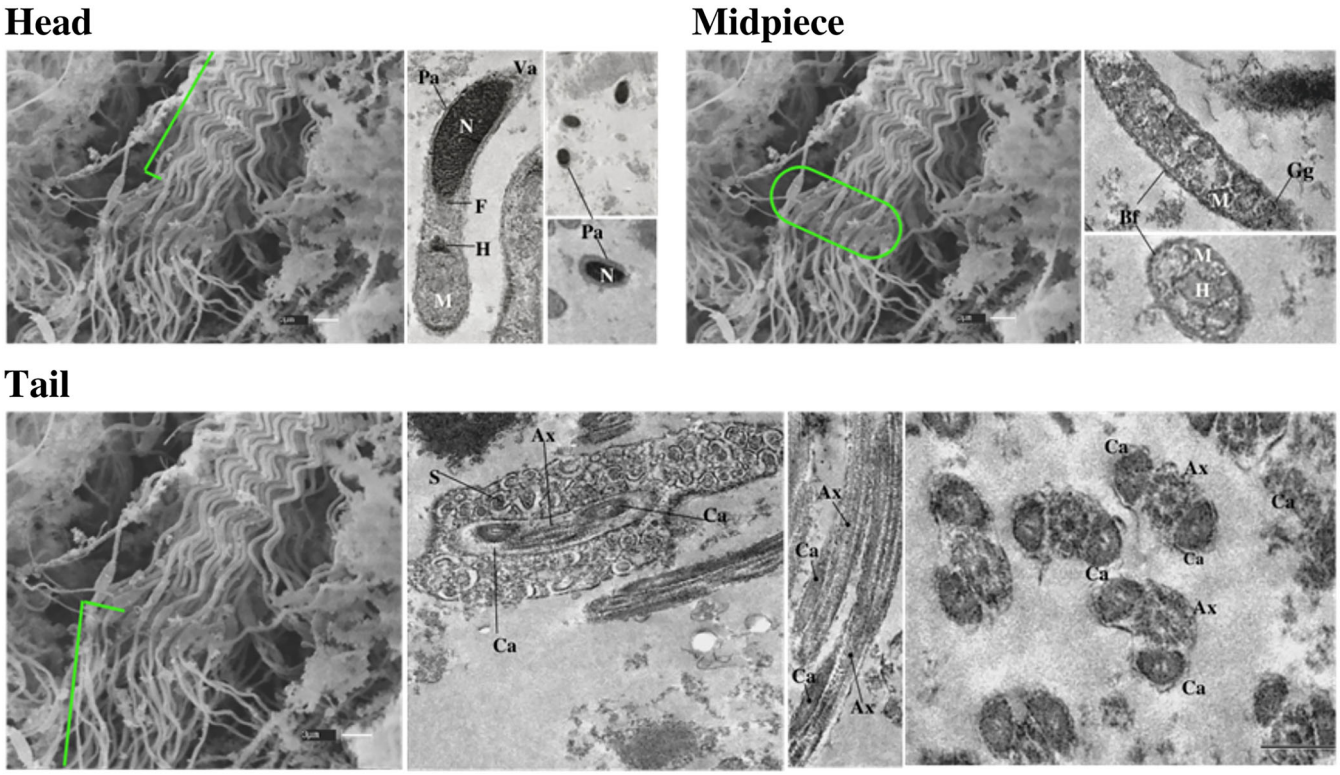

FIGURE 9 Scanning and transmission electron microscopy images of longitudinal and transverse sections of the sperm components of the shortnose guitarfish (*Zapteryx brevirostris*). Ax, axoneme; Ca, accessory axonemal columns; Bf, fibrous sheath of the midpiece; F, fossa nucleus asalsalis; Gg, glycogen granules; H, axial shaft of the midpiece; M, mitochondria in the midpiece; N, nucleus; Pa, parachromatin; S, cytoplasmic sleeve; Va, acrosomal vesicle

with the study conducted with *Hypanus americanus* (Hildebrand & Schroeder, 1928) (Gillis *et al*., 2021) but contrasts with research conducted with *C. taurus* (Wyffels *et al*., 2020) and *Chiloscyllium plagiosum* (Bennett, 1830) (Wyffels *et al*., 2020). In the case of the aforementioned shark species, there was a need for the addition of artificial salt water for initiation of sperm motility.

Further studies should seek to answer questions regarding the production of *Z. brevirostris* semen throughout the year and the variation in its concentration. Changes in semen quality may follow a seasonal reproductive pattern, as has been described for seasonal terrestrial species (Hofmann & Landeck, 1999). Understanding the seasonality of reproduction will aid in the management of the species

in zoos and aquaria by allowing managers to identify the best time of year to train voluntary semen collection, establish sexual maturity and assess reproductive capabilities, as well as diagnose reproductive abnormalities.

Through evaluation *via* light and electron microscopy, spermatozeugmata were found to be present in the semen of *Z. brevirostris*, that is, packets of sperm embedded in a matrix, showing alignment of their heads. The production of a strongly glycosylated matrix, along with other luminal secretions from Leydig's glands and contributions from the genital ducts, creates an environment that possibly protects and nourishes the sperm (Jamieson, 2005).

Sperm bundles are observed in the semen of many elasmobranch species (Pratt Jr & Tanaka, 1994; Wyfels *et al*., 2021) and may serve to minimize sperm loss during copulation, increase sperm storage efficiency in the male and female reproductive systems or preserve sperm longevity and motility during storage (Pratt Jr & Tanaka, 1994). The spermatozeugmata of *Z. brevirostris* are similar to the circular, matrix-containing aggregations of spermatozoa found in *Heterodontus portusjacksoni* (Meyer, 1793), *Raja eglanteria* (Bosc, 1802) and *Galeorhinus galeus* (Linnaeus, 1758) (Jones & Hamlett, 2006; Jones & Jones, 1982; McClusky, 2015) and markedly different from the laterally aligned sperm bundles observed in *Squalus acanthias* (Linneaus, 1758) and *Hydrolagus colliei* (Lay & Bennett, 1839) (Pratt Jr & Tanaka, 1994), or the complete envelopment of sperm by the luminal matrix to form spermatophores, as observed in *Cetorhinus maximus* (Gunnerus, 1765) (Matthews, 1950), *Callorhynchus mili* (Bory de Saint-Vincent, 1823) (Hamlett *et al*., 2002) and *C. taurus* (Wyffels *et al*., 2020).

The basic sperm morphology of *Z. brevirostris* is similar to that found in other species of chondrichthyans: (a) a helix-shaped head containing nucleus and acrosome, (b) a midpiece with the presence of mitochondria and (c) a tail containing axonema and accessory columns. To authors' knowledge, this article is the first to study scanning electron microscopic and transmission electron microscopic images of sperm in this species.

The head of most elasmobranch species, evaluated up to the publication date of this study, presented a helical shape. Nonetheless, in *Chlamydoselachus anguineus* (Garman, 1884), *Dalatias licha* (Bonnaterre, 1788) and *Squatina japonica* (Bleeker, 1858), the tip of the sperm head was observed to bend like a harpoon (Jamieson, 2005).

The nucleus has highly condensed and electrodense chromatin and is cone shaped, with a rounded posterior region and the presence of a depression, called the basal nuclear fossa, which accommodates the tip of the axial midpiece rod, the latter structure (axial rod) being unique to the chondrichthyan class within fishes (Jamieson, 2005; Temple-Smith *et al*., 2018). In the spermatozoa of *Z. brevirostris* the nuclear fossa has little marked delimitation, unlike species such as *Dasyatis kuhlii* (Müller & Henle, 1841) and *Hemitrygon fluviorum* (Ogilby, 1908), in which this fossa is pronounced (Jamieson, 2005).

Z. brevirostris and the other elasmobranch species studied to date have a shorter midpiece than the head (Jamieson, 2005). In contrast, all examined species of Holocephali have a long midpiece compared to the head (Jamieson, 2005). The midpiece may also be helically shaped, as in *Squalus suckleyi* (Linnaeus, 1758), or not, as in *Z. brevirostris* and *Scyliorhinus canicula* (Girard, 1855) (Muñoz-Baquero *et al*., 2021).

In rays and sharks, the microtubular arrangement of the axoneme can show a 9 + 2 or 9 + 0 pattern (Temple-Smith *et al*., 2018). In the case of the evaluated species, the pattern found was 9 + 2. To produce sperm motility, the axonema rotates along the length of the flagellum. In contrast, the longitudinal columns remain fixed in double positions 3 and 8 (Temple-Smith *et al*., 2018). Such features allow for the formation of a double-helix structure (Jamieson, 2005). Cylindrical rotatory movements, around its long axis, have also been described for spermatozoa from other ray species (Dzyuba *et al*., 2019), as well as from sharks (Minamikawa & Morisawa, 1996; Wyfels *et al*., 2020; Wyfels *et al*., 2021) and other vertebrates, suggesting that it is a movement performed for movement within the female's reproductive system and for fertilization.

The axoneme is abaxial not only in *Z. brevirostris* but also in *H. colliei* (Jamieson, 2005). Nonetheless, it lies along the central axis in *S. suckleyi* (Jamieson, 2005). The accessory axonemal columns in sharks *S. suckleyi* (Girard, 1854), *C. anguineus* (Garman, 1884), *Centroscymnus owstoni* (Garman, 1906), *Prionace glauca* (Lineu, 1758) and *Chiloscyllium punctatum* (Müller & Henle, 1838) are described as oval-shaped structures in cross-section, sometimes resembling the shape of a kidney, due to the fact that the median wall, being thinner than the lateral wall, can be indented (Jamieson, 2005). This same shape was identified in this study for *Z. brevirostris*. Nonetheless, the ray species *Himantura signifer* (Compagno & Roberts, 1982), *D. kuhlii* and *H. fluviorum* showed spermatozoa with the presence of two accessory axonemal columns that were either rounded (Chatchavalvanich *et al*., 2005; Jamieson, 2005).

The methodology used in this study fulfilled its aim of being useful in the evaluation of andrological parameters of *Z. brevirostris*. Investigations involving free-living shortnose guitarfish should be proposed, without, however, forgetting the fundamental role of aquaria and zoos as generators of knowledge. Given the threatened status of the species *Z. brevirostris* and considering the greater financial and logistical demands to make observations of animals *in situ*, longitudinal studies in zoos and aquaria are able to leverage the knowledge on reproductive aspects of the species and, consequently, contribute to the sustainability of the herds as well as to the management of wild populations. Therefore, institutions around the world that keep animals under human care should strengthen partnerships in the area of scientific research, contributing to the increase in sample sizes (*n*).

ACKNOWLEDGEMENTS

The authors thank the Ubatuba Aquarium and its technical staff for their support during the *ex situ* management and maintenance of the species *Z. brevirostris*.

CONFLICT OF INTEREST

The authors declare that they have no competing interests.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available from the corresponding author upon reasonable request.

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How to cite this article: de Oliveira Camilo, L., Maganhe, B. L., Gallo Neto, H., Crusco, S. E., Malavasi-Bruno, C. E., & Sanches, E. G. (2023). Andrology of shortnose guitarfish *Zapteryx brevirostris* (Müller & Henle, 1841) (Chondrichthyes, Trygonorrhinidae). *Journal of Fish Biology*, *103*(3), 635–645. https://doi.org/10.1111/jfb.15479